ISSN : 1812-5379 (Print)
ISSN : 1812-5417 (Online)
http://ansijournals.com/ja

JOURNAL OF AGRONOMY

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Journal of Agronomy

ISSN 1812-5379
DOI: 10.3923/ja.2017.23.31

Research Article
Changing of Morphological Characteristic and Biomass Properties in *Pennisetum purpureum* by Colchicine Treatment

[1]P. Kamwean, [1]T. Chaisan, [1]P. Thobunluepop, [1]C. Phumichai and [2]M. Bredemeier

[1]Department of Agronomy, Faculty of Agriculture, Kasetsart University, Thailand
[2]Forest Ecosystems Research Group, Center for Biodiversity and Sustainable Land Use (CBL), Universität Göttingen, Germany

Abstract

Background and Objective: Napier grass had limitation on conventional breeding program due to its self-incompatibility and sterilization effect. The fundamental goal of plant breeding program is to increase chemical composition and biomass yield of Napier grass by insure the yield per unit area and heating value for renewable energy utilization are also increased. In the experiment, the colchicine was applied to induce mutation in Napier grass cultivars in order to increase genetic variability of the varieties for good agricultural characteristics and future chemical component selection. The experiment was aimed to study the effect of colchicine treatment on the change of morphological characteristics, biomass properties and biomass yield of *Pennisetum purpureum*. **Materials and Methods:** The experiment was factorial in Complete Randomized Design (CRD) with 35 replications. There were four treatments accessions of *Pennisetum purpureum*, which were Chiang-Rai 2 (CR2), Chiang-Rai 3 (CR3), Taiwan A148 (TA148) and Tifton with five colchicine concentrations levels of 0.0, 0.05, 0.1, 0.2 and 0.3% (w/v) which was applied in each treatments. Colchicine was treated on shoot apical meristem of stem cutting. **Results:** The experiment found that colchicine treatment significantly affected on plant height, leaf greenness, stem diameter and stomatal size. The CR2, CR3 and Tifton after treated with 0.1, 0.2 and 0.3% (w/v) of colchicine had significantly increased on plant height, leaf biomass and biomass yield. The concentration of colchicine at 0.05% (w/v) significantly increased cellulose and lignin content of Tifton and CR2, respectively. Moreover, the colchicine treatment at 0.2% (w/v) significantly decrease ash content of Tifton. Furthermore, the flow cytometric histograms on DNA content showed the different between non-treated and colchicine treated samples. The concentration of colchicine at 0.1 and 0.2% (w/v) showed similarity pattern of aneuploidy cells. The CR3 at 0.1 and 0.2% (w/v) of colchicine and TA148 at 0.2% (w/v) of colchicine increase the DNA content compared to non-treated sample (the control). **Conclusion:** Finally, the colchicine treatment showed the improvement on plant morphological yield and yield components in the treated samples which was almost tends to show the dominant characteristic.

Key words: Energy crops, *Pennisetum purpureum*, colchicine treatment, morphological characteristics, biomass yield, biomass properties

Received: November 01, 2016 Accepted: December 01, 2016 Published: December 15, 2016

Citation: P. Kamwean, T. Chaisan, P. Thobunluepop, C. Phumichai and M. Bredemeier, 2017. Changing of morphological characteristic and biomass properties in *Pennisetum purpureum* by colchicine treatment. J. Agron., 16: 23-31.

Corresponding Author: T. Chaisan, Department of Agronomy, Faculty of Agriculture, Kasetsart University, Thailand

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The status of energy resources of Thailand currently have been reduced due to rapid industrial and commercial growth. Although, Thailand has some coal, lignite and natural gas reserves is available for only 12.5 years, while lignite is abundant for 100 years. Thailand almost import oil, coal, natural gas and electricity, which account for 46% of the total main energy consumption. In recent years, Thailand has been committed to improve energy security by promoting and supporting energy use efficiency and renewable energy utilization[1]. The utilization of biomass to generating heat and electricity was one of the potential of renewable energy focusing program in Thailand. Potential biomass sources in Thailand are divided into four groups, which are agricultural residues, agro-industrial residues from wood industries, furniture and energy crops. However, the agricultural residues and wasted had mentioned above still facing numerous problems, such as biomass insufficient quantity in some seasons, technically support and economic challenges[2]. The cultivation of biomass crops such as eucalyptus, cassava, maize, sugarcane and Napier grass becomes the alternative way to maintain long term production and sustainability of biomass feedstock to support to renewable energy utilization[3].

Pennisetum purpureum, commonly known as Napier grass belong to C4 plants which are fast-grown perennial grass and become more attractive for biomass energy production. Napier grass produced high biomass yield, high lignocellulose content, low input demand, fast regrowth after harvested and could be harvested continuously for 3-5 years[2]. However, the improvements of Napier biomass yield and their biomass properties were limited by environmental conditions, cultural practices and their genetically backup. The Napier grass breeding program was one way challenging to improve biomass yield and their biomass qualities. Napier grass is a short-day plant with low seed development and poor seed germination which limiting genetic improvement via conventional breeding program. The mutant induction might be the alternative technique for Napier genetically breeding improvement. Chemical treatment on shoot or auxiliary bud to induce ploidy levels or genetic variations might be the suitable method. Many studies *Salvia miltiorrhiza*[4], *Brassica rapa*[5], *Panax ginseng*[6], *Zingiber officinale*[7] and *Miscanthus* species[8], reported that colchicine treatment could enhance biomass yield and biomass properties. Colchicine is a tricyclic alkaloid substance which was applied to induce polyploidising and mutagenic agent[9]. It was inhibitors of mitosis process by binding to free tubulin with its topolone ring. The formation of microtubule and mitotic spindle were prevented[10] and then the cell plate formation which divides the cytoplasm into two parts during the cell division process was disturbed[11]. Kim *et al*.[6] found that colchicine treatments could induce polyploidy (octoploidy) in the adventitious roots of *P. ginseng* in order to enhance root biomass and ginsenoside accumulation. According to Glowacka *et al*.[8] reported that colchicine treatment induced polyploids characteristics which increase the stem diameter and dry matter weight of *Miscanthus* grass. Thus, the experiment was aimed to study the effect of colchicine treatment to induce ploidy level of elite varieties in order to change the morphology and biomass compositions of Napier grass.

MATERIALS AND METHODS

The experiment was factorial in Complete Randomized Design (CRD) with 35 replications. There were four accessions of Napier grass with five levels of the concentration of colchicine. The four accessions of *Pennisetum*s species include, Chaing-Rai 2 (CR2), Chaing-Rai 3 (CR3), Taiwan A148 (TA148) and Tifton. The accessions were provided by Vithai biopower company Ltd., Nakhon Ratchasima Animal Nutrition Research and Development Center and National Corn and Sorghum Research Center Thailand, respectively. Colchicine (97% analytical grade, Sigma Chemical Co.) was applied at the concentrations of 0, 0.05, 0.1, 0.2 and 0.3% (w/v). The stem cutting at 6 months old was incubated in 4×10 inch plastic bags containing soil and palm kernel cake mixture media (1:1, volume by volume, v/v) for 2 weeks. Then, four drops of colchicine treatments were applied on the auxiliary bud of incubated stem cutting, while distilled water was applied as control treatment. Then, cotton wool was place on the treated spots, which was covered by aluminum foil for 24 h to ensure colchicine should not degraded by environment factors. After 24 h of colchicine incubation the cotton wool and aluminum foil were removed. Then, control and treated samples were grown under greenhouse condition. Plants were watered by every 2 days interval with 1 L of water per treatment. The fertilizer was applied at the rate of 0.2 g (15-15-15 of N-P-K) per pot every 15 days. Morphological characteristics, biomass yield and biomass properties were recorded by 3 months intervals for 1 year as following.

Survival plants percentage: The survived plants were counted at 30 days after incubation. The survival percentage was calculated as compared to control according to Bashir[12] by the following equation:

$$\text{Survival plant percentage} = \frac{\text{No. of seedlings survived at 30 days after incubation}}{\text{No. of seedling of control}} \times 100$$

Plant height: The above ground main stem of each samples were measured for plant height.

Stem diameter: The stem diameters were measured by digital Vernier caliper on center of main stem and the result was expressed as millimeter.

Greenness value of leaves: The greenness values of leaves were measured by SPAD meter (SPAD-502Plus, KONICA MINOLTA). The measurement was taken on three leaves per plant, each leaf was measured at the middle of the leaf blade then the data were analysis for average of greenness value of leaves.

Stomatal size: Three months old leaf sample was taken for stomatal size evaluation. The lower epidermis of leaves was peeled off and placed on a slide glass and then a drop of water was added. Stomatal size was evaluated by 400x stereo microscope method (OLYMPUS CX21, AMERICA INC).

Chemical composition: Cellulose, lignin and ash content were analyzed by the FT-NIR (AgriQuant and infraQuant 2.5, Q-Interline A/S, Denmark). Samples were analyzed by the reflection measurement technique. The parameters were compared with the standard equation referenced by Maniin[13].

Cytological identification: The ploidy level and DNA content of control and treated samples were estimated by flow cytometric putative technique followed to Termkietpisan[14]. Fresh mature leaves samples were collected and section to approximately 0.1-0.5 g. Then, samples were stained by 1000 µL of cystain UV ploidy buffer (Cystain UV ploidy contained extraction buffer and staining buffer; DAPI dye) in a plastic petri dish. Obtained solution was filtered with a Partec CellTrics disposable filter to eliminate cell debris. Then, each sample was added 500 µL of cystain UV ploidy buffer. After that, the samples were analyzed using a Partec PAII laser flow cytometer (Partec GmbH, Münster, Germany). Histograms were analyzed using the Partec FloMax software, which determines the peak position, coefficient of variation and the relative ploidy index of the samples.

Statistical analysis: The analysis of variance (ANOVA) was used to analyze the significant of all collected data. Least Significant Difference (LSD) was used to analyze for mean comparison between treatments and their interaction, except flow cytometer data. The ANOVA was used to compare for significantly different at $p<0.05$. The data analyses were performed using R version 3.2.5 (2016-04-14) copyright (C) 2016 the R Foundation of Statistical computing platform: i386-w64-mingw32/i386 (32 bit).

RESULTS AND DISCUSSION

The increase in concentration of colchicine treatments affects the percentage rate of surviving plants. The maximum percentage of survival plant was found in control treatment, whereas the rate of survival was decreased gradually at the high concentration of colchicine. The lowest of survival percentage was noted at 0.3, 0.1 and 0.2% (w/v) of colchicine for CR2, CR3, TA148 and Tifton, respectively (Fig. 1). Colchicine could be infiltrate to inside cell that cause cytoplasm viscosity to make the abnormal cells[15]. According to Raphiphan[16] reported that the duration of soaking seeds and colchicine concentration had significant effects on the numbers of days required for total germination of *Ipomoea camellias* Linn. Addink[17] stated that high concentration of colchicine could inhibit the development of living part, which resulted in a mortality of organisms. The ability of colchicine to enter the cell of living organisms to interact with the DNA produces the general toxic effects associated with colchicine properties. Thus, their effects are mainly due to the direct interaction between the mutagen and the DNA molecular[18].

The colchicine treatments were significantly affected on greenness value of leaves. The control plant showed highest of leaf greenness value (34.5 SPAD unit) compared to colchicine treatment, the colchicine treatments significantly decreased leaf greenness value and the lowest value was found at 0.3% (w/v) of colchicine (32.84 SPAD unit) (Fig. 2).

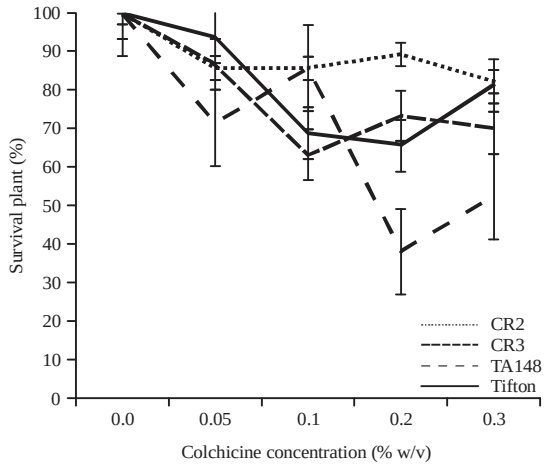

Fig. 1: Effect of various colchicine concentration survival percentage of treated plant samples

The reduction of leaf greenness value might be affected by colchicine inhibited some enzyme activity that affected to physiological expression[19].

Colchicine concentrations at 0.2, 0.1 and 0.3% (w/v) showed highest of plant height (98.9, 89.32 and 80.46 cm, respectively) that were significant different with control and 0.05% (w/v) of colchicine (71.07 and 72.66 cm, respectively) (Fig. 3).

The effect interaction of variety and colchicine concentrations on plant height, stem diameter and greenness value of leaves on TA148 at control showed the highest of plant height (122.4 cm) and were significant different when compare with other treatments (Table 1). The CR2 and CR3 at 0.1% (w/v) of colchicine showed higher plant height (107.6 and 101.1 cm, respectively) than other treatments within CR2 and CR3 varieties and significant different when

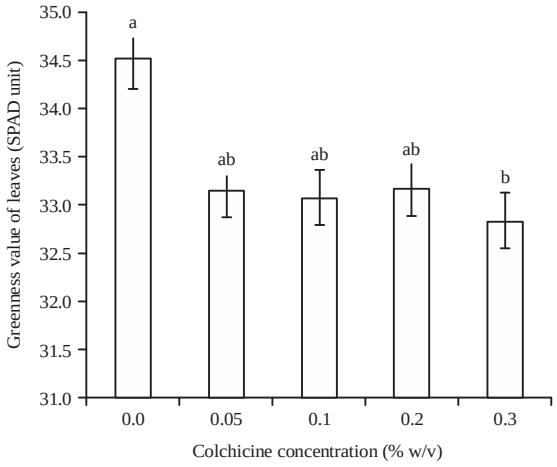

Fig. 2: Effect of colchicine concentration on greenness value of leaves of treated plant samples

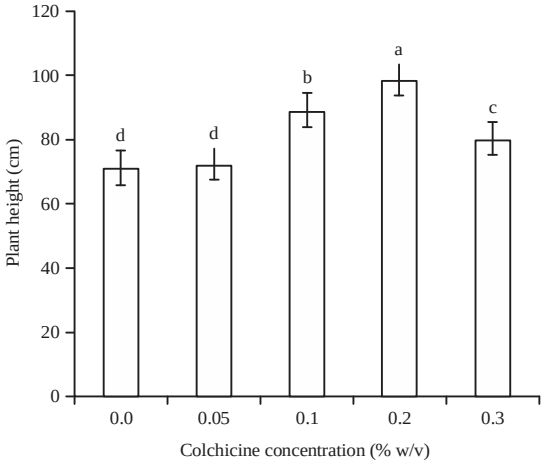

Fig. 3: Effect of colchicine concentration on plant height of treated plant samples

Table 1: Effect interaction of variety and colchicine concentrations on plant height, stem diameter and greenness value of leaves

Treatments		Data		
Varieties	Colchicine concentrate (%)	Plant height (cm)	Stem diameter (mm)	Greenness value of leaves (SPAD unit)
CR2	0.0	78.46^{def}	11.88^{cde}	34.35^{abcd}
	0.05	98.62^{bc}	13.07^{bc}	34.99^{abcd}
	0.1	107.60^{b}	13.87^{bc}	32.91^{cdefg}
	0.2	101.10^{bc}	12.21^{cde}	34.95^{abcd}
	0.3	97.43^{bc}	13.56^{bc}	30.94^{fg}
CR3	0.0	50.43^{g}	12.97^{bc}	35.1^{abc}
	0.05	67.96^{f}	13.53^{bc}	32.33^{defg}
	0.1	101.10^{bc}	14.26^{b}	35.74^{abc}
	0.2	98.23^{bc}	13.41^{bc}	32.85^{cdefg}
	0.3	71.80^{ef}	13.04^{bc}	28.72^{g}
TA148	0.0	122.40^{a}	16.41^{a}	31.71^{efg}
	0.05	87.60^{cd}	14.09^{b}	36.5^{ab}
	0.1	94.83^{bc}	12.45^{bcde}	33.31^{bcdef}
	0.2	84.67^{cdef}	13.97^{bc}	33.08^{bcdefg}
	0.3	85.40^{cde}	14.27^{b}	33.23^{bcdef}
Tifton	0.0	49.58^{g}	11.62^{def}	35.96^{b}
	0.05	48.50^{g}	11.58^{ef}	30.79^{fg}
	0.1	53.10^{g}	10.38^{f}	33.48^{bcdef}
	0.2	101.20^{bc}	14.13^{b}	31.55^{efg}
	0.3	70.19^{ef}	12.89^{bcd}	37.54^{a}
F-test		**	**	**
CV (%)		27.97	53.08	15.35

Values followed by different superscript letters within a column differ significantly at $p<0.05$

compare with control. Moreover, Tifton at 0.2% (w/v) of colchicine showed higher plant height (101.2 cm) than other treatments within the Tifton variety and significant different when compare with control. The decrease of plant height may resulted by the effect of colchicine treatment on physiological processes disturbances which causes the reduction in the cell division rate and resulted into slow of growth rate[20].

Furthermore, TA148 at control indicated highest stem diameter (16.41 mm) and significant different when compare other treatments followed by CR2 and CR3 at 0.1% (w/v) of colchicine with the stem diameter of 13.87 and 14.26 mm, respectively, however no significant different when compare with control in each the variety. Tifton at 0.2% (w/v) of colchicine showed higher of stem diameter (14.13 mm) than other treatments within the same variety and significant different when compare with control.

The observation shows that stem diameter increases with the increase in colchicine concentration[21]. The increase in dimensions and area were probably due to the fact that cells with a larger complement of chromosome grow larger to maintain a constant ratio of cytoplasmic to nuclear volume and express more proteins with the presence of more genes. This increase in size may translate to an increase in plant and its organs[22].

The result of greenness value of leaves found that Tifton at 0.3% (w/v) of colchicine showed higher of greenness value of leaves (37.54 SPAD unit) than other treatments and significant different when compare with control on Tifton but no significant different when compare with CR2 at control, 0.05 and 0.2%, CR3 at control and 0.1% and TA148 at 0.05% (w/v) of colchicine. Moreover, TA148 at 0.05% (w/v) of colchicine showed higher of greenness value of leaves (36.5 SPAD unit) than other treatment within same the variety and significant different when compare with control.

The content of chlorophyll was affected by environment conditions and genetic composition of the plant[18]. In the present studies found that the colchicine treatment significantly increased total chlorophyll content (Table 1). According with Kirhara[23] also reported that the leaves of polyploidy plants were large, thick and dark green than diploid plants. These finding is in agreed the results of Mensah *et al.*[18] for sesame (*Sesame indicum* L.), as well. Colchicine could be effects to basic set of gene that code for building blocks to give rise to an organism, that the change of pigments on the chlorophyll content.

The integral effect of Napier varieties and colchicine treatment on the length and width of stomata showed TA148 at 0.1% (w/v) and CR3 at 0.05% (w/v) of colchicine had greatest width of stomata 101.3 and 92.1 µm, respectively

Table 2: Influence of varieties and treatment combination on the length and width of stomata of treated plant material

	Width of stomata (µm)			
Colchicine concentration (%)	CR2	CR3	TA148	Tifton
Control	94.50^{ab}	59.33^{f}	81.77^{abcde}	73.05^{bcdef}
0.05	70.44^{def}	92.10^{abc}	-	64.50^{ef}
0.1	71.70^{def}	80.70^{abcdef}	101.30^{def}	63.65^{ef}
0.2	76.79^{bcdef}	85.08^{abcd}	71.40^{bcdef}	65.62^{ef}
0.3	82.07^{abcd}	71.74^{cdef}	77.13^{bcdef}	66.96^{ef}
Length of stomata (µm)				
Control	116.80^{abcd}	102.50^{cd}	123.00^{abc}	100.20^{cd}
0.05	107.10^{cd}	134.80^{ab}	-	112.40^{bcd}
0.1	105.40^{cd}	115.20^{abcd}	120.80^{abc}	93.60^{d}
0.2	121.30^{abc}	135.30^{a}	108.90^{bcd}	107.20^{cd}
0.3	124.80^{ab}	119.20^{abc}	110.90^{bcd}	107.00^{cd}

Values followed by different superscript letters within a column differ significantly at $p<0.05$

Table 3: Influence of varieties and treatment combination on biomass dry weight of treated plant material

	Difference in the biomass dry weight (g)			
Colchicine concentration (%)	CR2	CR3	TA148	Tifton
Control	56.25^{def}	70.00^{def}	77.00^{bcd}	67.00^{def}
0.05	76.67^{bcd}	63.33^{def}	63.33^{def}	50.00^{ef}
0.1	88.12^{abc}	79.38^{bcd}	68.85^{def}	44.38^{f}
0.2	70.00^{de}	101.90^{a}	42.50^{f}	71.82^{cde}
0.3	61.43^{def}	65.50^{def}	48.00e^{f}	89.06^{ab}

Values followed by different superscript letters within a column differ significantly at $p<0.05$

than any other treatment within the same variety and was significantly different when compare with control (Table 2). Furthermore, CR3 at 0.2% (w/v) of colchicine showed greatest length of stomata 135.3 µm than other treatment and significant different when compare with control.

It was found that control plants have differences in stomatal size that related to genetic of those plants. The results suggested that treated plants in CR3 and TA148 had fair amount of larger stomata at 0.05 and 0.1% (w/v) of colchicine, respectively. Stomatal size can be an indicator of ploidy level and it has been used in different plant type for determining ploidy levels[24-26]. In this study, morphological markers and stomata observations were used as a pre-screen for putative polyploidy from a larger population of treated plants. Subsequently, flow cytometry was used to confirm polyploidy status.

Table 3 showed the integral effect of variety and colchicine concentrations on biomass dry weight, it had been found that CR3 at 0.2% (w/v) of colchicine had higher biomass dry weight (101.9 g) than other treatment and significant different when compare with all treatments, except for Tifton at 0.3% (w/v) of colchicine. Moreover, CR2 at 0.1% (w/v) of colchicine showed higher biomass dry weight (88.12 g) than

Table 4: Influence of varieties and treatment combination on the change of chemical composition of treated plant material

Treatment		Data		
Varieties	Colchicine concentrate (%)	Cellulose	Lignin	Ash
CR2	0.0	45.94^{ab}	15.44^{abc}	9.602^{bcde}
	0.05	27.47^{cd}	17.79^{a}	9.636^{bcde}
	0.1	39.12^{abc}	16.99^{a}	7.597^{de}
	0.2	40.30^{abc}	15.28^{abc}	9.95^{bcde}
	0.3	39.07^{abc}	15.14^{abc}	9.272^{cde}
CR3	0.0	39.70^{abc}	15.08^{abc}	9.06^{cde}
	0.05	24.38^{d}	13.88^{bc}	12.28^{bc}
	0.1	29.60^{cd}	14.46^{bc}	10.69^{bcde}
	0.2	29.27^{cd}	15.83^{ab}	7.429^{de}
	0.3	38.53^{abc}	14.23^{bc}	10.26^{bcde}
TA148	0.0	45.87^{ab}	13.73^{bc}	11.53^{bcd}
	0.05	39.56^{abc}	12.70^{bcd}	14.48^{b}
	0.1	44.63^{ab}	14.04^{bc}	10.12^{bcde}
	0.2	44.59^{abc}	12.78^{bcd}	10.77^{bcde}
	0.3	24.85^{cd}	6.876^{e}	26.34^{a}
Tifton	0.0	47.33^{ab}	11.92^{cd}	13.27^{bc}
	0.05	49.27^{a}	13.03^{bc}	9.291^{bcde}
	0.1	34.84^{bcd}	12.48^{cd}	12.31^{bc}
	0.2	37.17^{bc}	15.12^{abc}	6.005^{e}
	0.3	26.6^{cd}	8.849^{de}	7.448^{de}
F-test		**	**	**
CV (%)		33.73	22.80	49.96

Values followed by different superscript letters within a column differ significantly at $p<0.05$

other treatments within the same variety and significant different when compare with control. Tifton at 0.3% (w/v) colchicine showed higher biomass dry weight (89.06 g) than other treatments within the same variety and significant different when compare with control.

The biomass dry weight corresponds with results of morphological characteristic in Table 1. The CR2, CR3 and Tifton varieties increased plant height, stem diameter and greenness value of leaves on treated plants and the total biomass dry weight (Table 3).

Influence of varieties and colchicine concentration on the change of chemical composition has been determined after screen biomass dry weight. For this study we interested in cellulose, lignin and ash content that these chemical composition is responsible for heating value in combustion.

The study interaction between varieties and colchicine concentration on the change of chemical composition of treated plants found that Tifton at 0.05% (w/v) of colchicine indicated that higher cellulose content (49.27% of cellulose) than other treatment but no significant different when compare with control. The CR2 at 0.05% (w/v) of colchicine showed highest lignin content (17.79% of lignin) but no significant different when compare with control. Moreover, Tifton at 0.2% (w/v) of colchicine showed higher lignin content (15.12% of lignin) than other treatment within the Tifton variety but no significant different when compare with control. Furthermore, Tifton at 0.2% (w/v) of colchicine showed lowest of ash content (6.05%) and it was significant different when compare with control (Table 4).

The concentration of colchicine at 0.1 and 0.2% (w/v) increased DNA content of CR3 (431.33 and 435.26, respectively) when compared to control plant (408.55). The treated plants were showed aneuploidy, while control plant showed tetraploid characteristic. Moreover, colchicine treatment also increased DNA content of TA148. Colchicine concentration at 0.1% (w/v) with DNA content of 430.21 (aneuploid), which was higher than these of control plant (409.20, tetraploid) (Table 5, Fig. 4). The mutant samples were showed dominant characteristic which provide high number of node, length of leaf blade, plant height and stem diameter than non-mutant sample. Interestingly, ash content of mutant plants was less than non-mutant sample. The low ash content in biomass indicates an important to use Napier grasses for biomass energy. Ash includes the elements which are most abundant in the fuel like Al, Ca, Fe, Mg, P, K, Si, Na and Ti. These elements had a major impact on the ash melting behavior, slagging, fouling and corrosion of power plant system. High ash content tends to decrease the efficiency of the combustion system and increase the operation and maintenance cost. On the other hand, high concentrations of some elements may indicate fuel contamination with soil or sand[27].

Edme *et al.*[28] found that DNA content was positively and significantly correlated with plant height, clones with a larger amount of DNA tends to be taller. Likewise according Yan *et al.*[29] suggested that the DNA content was positively correlated with plant height, stem diameter, leaf width and dry weight. Moreover, Bennett and Leitch[30] reported that variation in nuclear DNA content had a major impact on many plant traits. The aneuploidy was abnormal numbers of colchicine, where an extra or missing chromosome can introduce genetic disorder. The aneuploidy happened during cell division and only single chromosome are affected not the whole genome[14,31]. It could also be possible that centromeres interacted unequally with the mitotic spindle, causing chromosome loss[14,32]. Chromosome doubling is widely effected by colchicine but the efficiency of colchicine treatments seems to depend up on the genotype, temperature, quality and intensity of light and all growing environmental conditions and therefore, the aneuploidy effects on gene expression and cell physiology. The different species and even different individuals of the same species, respond differently to colchicine. Immunity of colchicine system was referenced by Cornman[33] that reside in

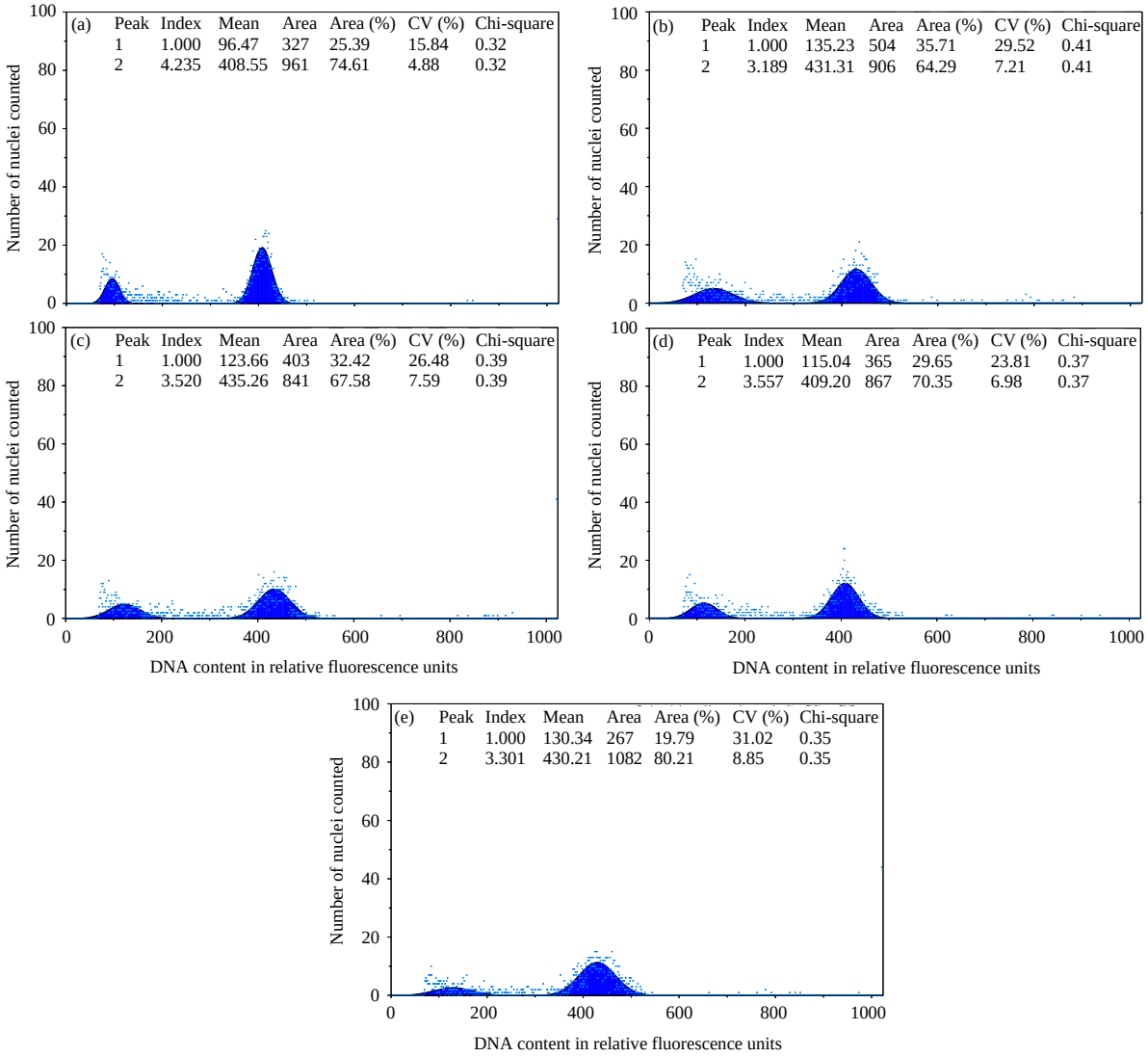

Fig. 4(a-e): Flow cytometric histogram of DNA content of *Pennisetum* species from treated with colchicine (a) Control of CR3, (b) CR3 at 0.1%, (c) CR3 at 0.2%, (d) Control of TA148 and (e) TA148 at 0.1%

Table 5: Peaks and ploidy at flow cytometric determination according to Fig. 4

Varieties	Colchicine concentration (%)	Mean of peak	Ploidy (n) indicated by channel number
CR3	Control	408.55	Tetraploid (4n)
	0.1	431.33	Aneuploidy between 4n and 5n
	0.2	435.26	Aneuploidy between 4n and 5n
TA148	Control	409.20	Tetraploid (4n)
	0.1	430.21	Aneuploidy between 4n and 5n

"Some extra mitotic protective mechanism" cell which have been treated with colchicine often revert to original ploidy cell which "was not divided" at the time of treatment. According to Ascough and Van Staden[34], tested *Watsonia lepida* under colchicine treatment for 24 h with lowest concentration, but no polyploidy plants were found, but at 48 h treatment produce polyploidy plant where as further application time at 72 h, mixoploidy occurred. Shoots on older plants can be treated, but it is often less successful and resulted in a greater percentage of cytochimeras[14,35]. Axillary or sub-axillary meristems are usually induced for orchid and chemical solutions can be applied to buds using cotton, agar or lanolin or by dipping branch tips into a solution for a few hours or days[35,36].

CONCLUSION

Different concentration of colchicine treatment on *Pennisetum purpureum* had effect on plant morphological characteristics and biomass properties. The CR2, CR3 and Tifton when treated with 0.1-0.3% of colchicine the observation indicate an increase of plant height, leaf biomass and biomass yield. When 0.05% concentration of colchicine was used there was the increase in cellulose and lignin content for Tifton and CR2, respectively. The appearance of aneuploidy cell on CR3 and TA148 aneuploidy plants increased DNA content that has shown agronomical dominant characteristics. Finally, the induced mutation of different colchicine concentration in Napier grass varieties has increase the genetic, morphological variability and chemical component for the TA148, CR2, CR3 and Tifton varieties, respectively.

ACKNOWLEDGMENTS

The authors would like first to acknowledgement Graduate School of Kasetsart University 72 years anniversary for the provision of research fund. Secondly gratefully thanks should go to the Vithai Bio Power Company and National Corn and Sorghum Research Center for plant material. And finally to the Faculty of Agriculture, Kasetsart University for resources, generous place and equipment for research achievements.

REFERENCES

1. GNESD., 2010. Energy security in Thailand. Asian Institute of Technology School of Environment, Resources and Development, Thailand.
2. Waramit, N. and J. Chaugool, 2014. Napier grass: A novel energy crop development and the current status in Thailand. J. Int. Soc. Southeast Asian Agric. Sci., 20: 139-150.
3. DAEDE., 2012. Annual report 2012. Department of Alternative Energy Development and Efficiency, Ministry of Energy, Thailand.
4. Gao, S.L., D.N. Zhu, Z.H. Cai and D.R. Xu, 1996. Autotetraploid plants from colchicine-treated bud culture of Salvia miltiorrhiza Bge. Plant Cell Tissue Organ Cult., 47: 73-77.
5. Abel, S. and H.C. Becker, 2007. The effect of autopolyploidy on biomass production in homozygous lines of *Brassica rapa* and *Brassica oleracea*. Plant Breed., 126: 642-643.
6. Kim, Y.S., E.J. Hahn, H.N. Murthy and K.Y. Paek, 2014. Adventitious root growth and ginsenoside accumulation in *Panax ginseng* cultures as affected by methyl jasmonate. Biotechnol. Lett., 26: 1619-1622.
7. Wei, K.H., J.H. Miao, H.P. Huang and S.L. Gao, 2011. Generation of autotetraploid plant of ginger (*Zingiber officinale* Rosc.) and its quality evaluation. Pharmacogn. Mag., 7: 200-206.
8. Glowacka, K., S. Jezowski and Z. Kaczmarek, 2009. Polyploidization of *Miscanthus sinensis* and *Miscanthus x giganteus* by plant colchicine treatment. Ind. Crops Prod., 30: 444-446.
9. Bragal, M., 1955. Production of polyploids by colchicine. Euphytica, 4: 76-82.
10. Metzler, D.E. and C.M. Metzler, 2003. Biochemistry: The Chemical Reactions of Living Cells. Academic Press, San Diego, CA., USA., ISBN: 9780124925410, Pages: 1973.
11. Klug, W.S. and M.R. Cummings, 2003. Genetics: A Molecular Perspective. Pearson Education Inc., Upper Saddle River, NJ., USA., Pages: 691.
12. Bashir, S., 2012. Studies on the induction of mutations in fenugreek (*Trigonella foenum-graecum* L.). Master Thesis, University of Kashmir, Srinagar, Hazratbal.
13. Maniin, P., 2015. Cutting interval of 7 tropical grasses on growth biomass yield and biogas productivity. M.Sc. Thesis, Kasetsart University, Bangkok, Thailand.
14. Termkietpisan, W., 2013. Effects of different colchicine concentrations on latex amount and changes in certain stomata characteristics of *Hevea brasiliensis* Muell. Arg. seedlings *in vivo*. Kasetsart J. (Nat. Sci.), 47: 311-322.
15. Cook, J.W. and J.D. Loudon, 1952. Colchicine. In: Alkaloids, Manske, R.H.F. and H.L. Holmes (Eds.)., Vol. 2, Academic Press, New York, USA., pp: 261-329.
16. Raphiphan, P., 2000. The effects of colchicine concentration and treated time on germination, growth and chromosome number in some potential ornamental plant. M.Sc. Thesis, Chiang Mai University. Chiang Mai, Thailand.
17. Addink, W., 2002. Colchicine used in plant breeding work to induce mutations (Polyploidy). http://biotech.icmb.utexas.edu/botany/calch.html
18. Mensah, J.K., B.O. Obadoni, P.A. Akomeah, B. Ikhajiagbe and J. Ajibolu, 2007. The effects of sodium azide and colchicine treatments on morphological and yield traits of sesame seed (*Sesame indicum* L.). Afr. J. Biotechnol., 6: 534-538.
19. Lu, Y., J. Chen, M. Xiao, W. Li and D.D. Miller, 2012. An overview of tubulin inhibitors that interact with the colchicine binding site. Pharmaceut. Res., 29: 2943-2971.
20. Swanson, C.P., 1957. Cytology and Cytogenetics. Prentice-Hall, Englewood Cliffs, NJ., USA., Pages: 596.
21. Wu, Y.Q. and M. Zhang, 2012. Effects on polyploidy induction in asparagus lettuce of concentration and presoaking time of colchicine. Agric. J., 7: 177-179.
22. Rauf, S., I.A. Khan and F.A. Khan, 2006. Colchicine-induced tetraploidy and changes in allele frequencies in colchicine-treated populations of diploids assessed with RAPD markers in *Gossypium arboreum* L. Turk. J. Biol., 30: 93-100.

23. Kirhara, H., 1951. Triploid watermelons. Proc. Am. Soc. Hort. Sci., 58: 217-230.
24. Moghbel, N., M.K. Borujeni and F. Bernard, 2015. Colchicine effect on the DNA content and stomata size of *Glycyrrhiza glabra* var. *glandulifera* and *Carthamus tinctorius* L. cultured *in vitro*. J. Genet. Eng. Biotechnol., 13: 1-6.
25. Huang, H., S. Gao, D. Wang, P. Huang and J. Li, 2014. Autotetraploidy induced in nianmaohuangqin (*Radix scutellariae viscidulae*) with colchicine *in vitro*. J. Tradit. Chin. Med., 34: 199-205.
26. Hamill, S.D., M.K. Smith and W.A. Dodd, 1992. *In vitro* induction of banana autotetraploids by colchicine treatment of micropropagated diploids. Austr. J. Bot., 40: 887-896.
27. Bisyplan Handbook, 2012. The ash composition. http://bisyplan.bioenarea.eu/ash_appendix.pdf
28. Edme, S.J., J.C. Comstock, J.D. Miller and P.Y.P. Tai, 2005. Determination of DNA content and genome size in sugarcane. J. Am. Soc. Sugar Cane Technol., 25: 1-16.
29. Yan, J., J. Zhang, K. Sun, D. Chang and S. Bai *et al*., 2016. Ploidy level and DNA content of *Erianthus arundinaceus* as determined by flow cytometry and the association with biological characteristics. PLOS One, Vol. 11. 10.1371/journal.pone.0151948.
30. Bennett, M.D. and I.J. Leitch, 1997. Nuclear DNA amounts in angiosperms-583 new estimates. Ann. Bot., 80: 169-196.
31. Sitto, P., E.W. Weiler, J.W. Kadereit, A. Bresinsky and C. Korner, 2002. Straburger-Lehrbuch der Botanik. Spektrum Akademuscher Verlag, Heidelberg, Germany, Pages: 1123.
32. Ravi, M. and S.W.L. Chan, 2010. Haploid plants produced by centromere-mediated genome elimination. Nature, 464: 615-618.
33. Cornman, I., 1941. Disruption of mitosis in Colchicum by means of colchicine. Biol. Bull., 81: 297-298.
34. Ascough, G.D. and J. van Staden, 2008. Effectiveness of colchicine and oryzalin at inducing polyploidy in *Watsonia lepida* N.E. Brown. HortScience, 43: 2248-2251.
35. Ranney, T.G. and J.R. Jones, 2008. Understanding Polyploidy: Insights into the evolution and breeding of azaleas. The Azalean/Winter, April 28, 2008, pp: 81-84.
36. Wu, Y.X., F.H. Yang, X. Zhao and W. Yang, 2011. Identification of tetraploid mutants of *Platycodon grandiflorus* by colchicine induction. Caryologia, 64: 343-349.